

which hybridizes under at least low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand, wherein the variant has chymotrypsin-like activity and has an amino acid sequence that has at least 70% identity to the amino acid sequence of the microbial trypsin.

2-23. (Cancelled)

24. (Original) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P.

25-31. (Cancelled)

32. (Original) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; and one or more deletions selected from the group consisting of V192*, K197*, and A226*.

33-35. (Cancelled)

36. (Original) The variant of claim 1, which comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; one or more deletions selected from the group consisting of V192*, K197*, and A226*; and the insertion G224GT of amino acids 25 to 248 of SEQ ID NO: 2.

37-51. (Cancelled)

52. (Original) The variant of claim 1, wherein the total number of substitutions is 11, more preferably 10, even more preferably 9, even more preferably 8, even more preferably 7, even more preferably 6, even more preferably 5, even more preferably 4, even more preferably 3, even more preferably 2, and most preferably 1.

53. (Original) The variant of claim 1, wherein the total number of deletions is 3, more preferably 2, and most preferably 1.

54-59. (Cancelled)

60. (Original) The variant of claim 1, which is encoded by the nucleotide sequence contained in pEJG66.1XLGOLD which is contained in *E. coli* NRRL B-30627.

61. (Original) The variant of claim 1, which is in the form of a precursor comprising amino acids 1 to 24 of SEQ ID NO: 2 as the prepro region, or a portion thereof, linked in translation reading frame with the amino terminus of the trypsin variant.

62. (Original) An isolated nucleotide sequence encoding the variant of claim 1.

63. (Original) An expression vector comprising the nucleotide sequence of claim 62.

64. (Original) A host cell comprising the expression vector of claim 63.

65-67. (Cancelled)

68. (Original) A method for obtaining a variant of a microbial trypsin, comprising:

(a) introducing one or more modifications selected from the group consisting of:

(1) a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2,

(2) a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and

(3) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2;

wherein the microbial trypsin is (a) a polypeptide having an amino acid sequence which has at least 70% identity to amino acids 25 to 248 of SEQ ID NO: 2; or (b) a polypeptide encoded by a nucleotide sequence which hybridizes under low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand, wherein the variant has microbial trypsin activity and has an amino acid sequence that has at least 70% identity to the amino acid sequence of the

microbial trypsin; and

(b) recovering the variant having chymotrypsin-like activity.

69. (Original) A method for producing a microbial trypsin variant, comprising:

(a) cultivating a host cell under conditions suitable for the expression of the variant, wherein the host cell comprises a nucleotide sequence encoding the variant comprising one or more modifications selected from the group consisting of:

(a) a substitution at one or more positions corresponding to positions 144, 193, 198, 201, 218, 223, 227, 228, 229, 230, and 231 of amino acids 25 to 248 of SEQ ID NO: 2,

(b) a deletion at one or more positions corresponding to positions 192, 197, and 226 of amino acids 25 to 248 of SEQ ID NO: 2; and

(c) an insertion between positions corresponding to positions 224 and 225 of amino acids 25 to 248 of SEQ ID NO: 2;

wherein the microbial trypsin is (a) a polypeptide having an amino acid sequence which has at least 70% identity to amino acids 25 to 248 of SEQ ID NO: 2; or (b) a polypeptide encoded by a nucleotide sequence which hybridizes under low stringency conditions with nucleotides 202 to 801 of SEQ ID NO: 1 or its complementary strand, wherein the variant has microbial trypsin activity and has an amino acid sequence that has at least 70% identity to the amino acid sequence of the microbial trypsin; and

(b) recovering the variant from the cultivation medium.

70-91. (Cancelled)

92. (Original) The method of claim 69, wherein the variant comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P.

93-99. (Cancelled)

100. (Original) The method of claim 69, wherein the variant comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; and one or more deletions selected from the group consisting of V192*, K197*, and

A226*.

101-103. (Cancelled)

104. (Original) The method of claim 69, wherein the variant comprises one or more substitutions selected from the group consisting of V144T, S193A, D198S, Q201M, A218I, N223S, R227S, P228T, N229S, Y230T, and S231P; one or more deletions selected from the group consisting of V192*, K197*, and A226*; and the insertion G224GT of amino acids 25 to 248 of SEQ ID NO: 2.

105-119. (Cancelled)

120. (Original) The method of claim 69, wherein the total number of substitutions is 11, more preferably 10, even more preferably 9, even more preferably 8, even more preferably 7, even more preferably 6, even more preferably 5, even more preferably 4, even more preferably 3, even more preferably 2, and most preferably 1.

121. (Original) The method of claim 69, wherein the total number of deletions is 3, more preferably 2, and most preferably 1.

122-127. (Cancelled)

128. (Original) The method of claim 69, wherein the variant is encoded by the nucleotide sequence contained in pEJG66.1XLGOLD which is contained in *E. coli* NRRL B-30627.

129. (Original) The method of claim 69, wherein the variant is in the form of a precursor comprising amino acids 1 to 24 of SEQ ID NO: 2 as the prepro region, or a portion thereof, linked in translation reading frame with the amino terminus of the trypsin variant.

130. (Original) A detergent composition comprising a variant of claim 1 and a surfactant.